

## **AMENDMENTS TO THE CLAIMS**

### **LISTING OF CLAIMS:**

Claim 1. (Withdrawn) A cytochrome *c* oxidase complex having cytochrome *c* oxidase activity, which complex is obtainable by the isolation from a *Gluconobacter oxydans* DSM 4025 microorganism.

Claim 2. (Withdrawn) A cytochrome *c* oxidase complex according to claim 1, wherein the microorganism is a biologically and/or taxonomically homogeneous culture of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.

Claim 3. (Withdrawn) A cytochrome *c* oxidase complex according to claim 1, wherein the complex has the following properties:

(a) comprising at least two core subunits of I (COI) and II (COII), wherein the apparent molecular mass of COI and COII are about 43  $\pm$  10 kDa and 36  $\pm$  10 kDa, respectively by SDS-PAGE; and

(b) providing an absorption spectrum showing an *aa3*-type cytochrome *c* oxidase peak at 605  $\pm$  1 nm in reduced minus oxidized difference spectrum.

Claim 4. (Withdrawn) A cytochrome *c* oxidase complex according to claim 1, wherein the isolated complex is substantially homologous to a native cytochrome *c* complex from *Gluconobacter oxydans* DSM 4025 or a biological or taxonomic homolog of a

microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.

Claim 5. (Withdrawn) A cytochrome *c* oxidase complex according to any one of claims 1-4, which is a recombinant enzyme.

Claim 6. (Withdrawn) A cytochrome *c* oxidase complex according to claim 5 comprising a core subunit containing the amino acid sequence of SEQ ID NO: 2.

Claim 7. (Withdrawn) A cytochrome *c* oxidase complex according to claim 6 comprising an amino acid sequence having 85% or greater sequence identity with SEQ ID NO: 2, and having cytochrome *c* oxidase activity.

Claim 8. (Withdrawn) A cytochrome *c* oxidase complex according to claim 5 comprising at least one amino acid sequence selected from the group of SEQ ID NO: 4, 6 or 8.

Claim 9. (Withdrawn) A cytochrome *c* oxidase complex according to claim 8, wherein the amino acid sequence is at least 85% identical to SEQ ID NO: 4, 6 or 8, and is capable of providing the complex with cytochrome *c* oxidase activity.

Claim 10. (Withdrawn) A recombinant polypeptide comprising an amino acid sequence of SEQ ID NO: 2.

Claim 11. (Withdrawn) A recombinant polypeptide according to claim 10, wherein the amino acid sequence is at least 85% identical to SEQ ID NO: 2, and is capable of providing the complex described in any one of claims 1 - 9 with cytochrome c oxidase activity.

Claim 12. (Withdrawn) A recombinant polypeptide according to claim 10, which is encoded by the polynucleotide sequence of SEQ ID NO: 1.

Claim 13. (Withdrawn) A recombinant polypeptide according to claim 12, wherein the polynucleotide sequence encodes SEQ ID NO: 2 or an amino acid sequence having at least 85% identity with SEQ ID NO: 2 and being capable of providing the complex with cytochrome c oxidase activity.

Claim 14. (Withdrawn) A recombinant polypeptide comprising an amino acid sequence of SEQ ID NO: 4.

Claim 15. (Withdrawn) A recombinant polypeptide according to claim 14, wherein the polypeptide has an amino acid sequence that is at least 85% identical to SEQ ID NO: 4, and is capable of providing the complex described in any one of claims 1 - 9 with cytochrome c oxidase activity.

Application No.: 09/712,768  
Amendment Dated: November 11, 2000  
Reply to Office Action mailed: February 7, 2003

Claim 16. (Withdrawn) A recombinant polypeptide according to claim 14, which is encoded by a polynucleotide sequence of SEQ ID NO: 3.

Claim 17. (Withdrawn) A recombinant polypeptide according to claim 16, wherein the polynucleotide encodes SEQ ID NO: 4 or an amino acid sequence having at least 85% identity with SEQ ID NO: 4 and being capable of providing the complex in any one of claims 1 - 9 with cytochrome c oxidase activity.

Claim 18. (Withdrawn) A recombinant polypeptide comprising an amino acid sequence of SEQ ID NOs: 6 or 8.

Claim 19. (Withdrawn) A recombinant polypeptide according to claim 18, wherein the amino acid sequence is at least 85% identical to either SEQ ID NOs: 6 or 8, and is capable of providing the complex described in any one of claims 1 - 9 with cytochrome c oxidase activity.

Claim 20. (Withdrawn) A recombinant polypeptide according to claim 18, which is encoded by a polynucleotide selected from the group consisting of SEQ ID NO: 5 and SEQ ID NO: 7.

Claim 21. (Withdrawn) A recombinant polypeptide according to claim 20 capable of providing the complex in any one of claims 1 - 9 with cytochrome c oxidase activity, which is encoded by a polynucleotide selected from the group consisting of a

polynucleotide encoding SEQ ID NO: 6, a polynucleotides encoding SEQ ID NO: 8, a polynucleotide encoding a polypeptide that is at least 85% identical to SEQ ID NO: 6, and a polynucleotide encoding a polypeptide that is at least 85% identical to SEQ ID NO: 8.

Claim 22. (Currently amended) A recombinant DNA ~~polynucleotide fragment~~ comprising the polynucleotide sequence of SEQ ID NO: 1.

Claim 23. (Currently amended) A recombinant DNA ~~polynucleotide fragment~~ comprising a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 2.

Claim 24. (Currently amended) A recombinant DNA ~~that encodes at least a part of core subunit I of a cytochrome c oxidase complex and that conveys cytochrome c oxidase activity to the complex when present, the recombinant DNA~~ comprising a polynucleotide sequence that encodes a polypeptide having an amino acid sequence that is at least 85% identical to SEQ ID NO: 2, wherein the polypeptide forms a complex having cytochrome c oxidase activity with a *Gluconobacter oxydans* cytochrome c oxidase core subunit II.

Claim 25. (Currently amended) A recombinant DNA ~~that encodes at least a part of core subunit II of a cytochrome c oxidase complex and that conveys cytochrome c oxidase activity to the complex when present, the recombinant DNA~~ comprising SEQ ID

NO: 3, wherein the recombinant DNA encodes at least a part of a *Gluconobacter oxydans* cytochrome c oxidase subunit II that forms a complex having cytochrome c oxidase activity with a *Gluconobacter oxydans* cytochrome c oxidase core subunit I.

Claim 26. (Currently amended) A recombinant DNA ~~that encodes at least a part of core subunit II of a cytochrome c oxidase complex and that conveys cytochrome c oxidase activity to the complex when present,~~ the recombinant DNA comprising a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 4, wherein the recombinant DNA encodes at least a part of a *Gluconobacter oxydans* cytochrome c oxidase subunit II that forms a complex having cytochrome c oxidase activity with a *Gluconobacter oxydans* cytochrome c oxidase core subunit I.

Claim 27. (Cancelled).

Claim 28. (Currently amended) A recombinant DNA ~~that encodes at least a part of core subunit III of a cytochrome c oxidase complex and that conveys cytochrome c oxidase activity to the complex when present,~~ the recombinant DNA comprising SEQ ID NOs: 5 or 7, wherein the recombinant DNA encodes at least a part of a *Gluconobacter oxydans* cytochrome c oxidase subunit III that forms a complex having cytochrome c oxidase activity with *Gluconobacter oxydans* cytochrome c oxidase core subunits I and II.

Claim 29. (Currently amended) A recombinant DNA ~~that encodes at least a part of core subunit III of a cytochrome c oxidase complex and that conveys cytochrome c oxidase activity to the complex when present,~~ the recombinant DNA comprising a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NOs: 6 or 8, wherein the recombinant DNA encodes at least a part of a *Gluconobacter oxydans* cytochrome c oxidase subunit III that forms a complex having cytochrome c oxidase activity with *Gluconobacter oxydans* cytochrome c oxidase core subunits I and II.

Claim 30. (Cancelled).

Claim 31. (Currently amended) An expression vector comprising a recombinant DNA according to any one of claims 22-26, 28 and 29 ~~that encodes at least a part of core subunits I, II and III of a cytochrome c oxidase complex and that conveys cytochrome c oxidase activity to the complex when present,~~ the recombinant DNA comprising a polynucleotide sequence ~~selected from the group consisting of polynucleotide sequences encoding the amino acid sequences of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8,~~ wherein the expression vector is suitable for expression in an organism.

Claim 32. (Cancelled).

Claim 33. (Original) An expression vector according to claim 31, wherein the organism is a microorganism.

Claim 34. (Original) An expression vector according to claim 33, wherein the microorganism is a bacteria.

Claim 35. (Original) An expression vector according to claim 34, wherein the bacteria is selected from the group consisting of *Escherichia coli*, *Pseudomonas putida*, *Acetobacter xylinum*, *Acetobacter pasteurianus*, *Acetobacter aceti*, *Acetobacter hansenii*, and *Gluconobacter oxydans*.

Claim 36. (Currently amended) An expression vector according to claim 35, wherein ~~wherein~~ the bacteria is *Gluconobacter oxydans* DSM 4025.

Claim 37. (Previously presented) An expression vector according to claim 34, wherein the bacteria is a biologically and/or taxonomically homogeneous culture of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.

Claim 38. (Original) A recombinant microorganism comprising the expression vector of claim 31.

Claim 39. (Original) A recombinant microorganism comprising the expression vector of claim 36.



Claim 40. (Currently amended) A recombinant microorganism comprising at least one recombinant DNA according to any one of claims 22-26, 28 and 29 ~~that encodes at least a part of core subunits I, II and III of a cytochrome c oxidase complex and that conveys cytochrome c oxidase activity to the complex when present, the recombinant DNA comprising a polynucleotide sequences selected from the group consisting of a polynucleotide sequence of SEQ ID NO: 1, a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 2, a polynucleotide sequence of SEQ ID NO: 3, a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 4, a polynucleotide sequence of SEQ ID NO: 5, a polynucleotide sequence that encodes the amino acid of SEQ ID NO: 6, a polynucleotide sequence of SEQ ID NO: 7, a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 8, and combinations thereof.~~

Claim 41. (Cancelled).

Claim 42. (Original) A recombinant microorganism according to claim 40, wherein the microorganism is a bacteria.

Claim 43. (Currently amended) A recombinant microorganism according to claim 40 42, wherein the microorganism is selected from the group consisting of *Escherichia coli*, *Pseudomonas putida*, *Acetobacter xylinum*, *Acetobacter pasteurianus*, *Acetobacter aceti*, *Acetobacter hansenii*, and *Gluconobacter oxydans*.

Claim 44. (Currently amended) A recombinant microorganism according to claim 40 43, wherein the microorganism is obtained from *Gluconobacter oxydans* DSM 4025.

Claim 45. (Previously presented) A recombinant microorganism according to claim 40, wherein the microorganism is a biologically and/or taxonomically homogeneous culture of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.

Claim 46. (Currently amended) A process for producing a cytochrome c oxidase complex comprising:

(a) cultivating in a culture medium a recombinant microorganism according to claim 40 ~~comprising at least one recombinant DNA that encodes at least a part of core subunits I, II and III of a cytochrome c oxidase complex and that conveys cytochrome c oxidase activity to the complex when present, the recombinant DNA comprising polynucleotide sequences selected from the group consisting of SEQ ID NO: 1, a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 2, a polynucleotide sequence that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 3, a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 4, a polynucleotide sequence that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 5, a polynucleotide sequence that encodes the amino acid of SEQ ID NO: 6, a polynucleotide sequence that encodes an amino acid sequence that is at least 85% identical to the amino acid~~

~~sequence of SEQ ID NO: 6, SEQ ID NO: 7, a polynucleotide sequence that encodes the amino acid sequence of SEQ ID NO: 8, a polynucleotide sequence that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 8, and combinations thereof; and~~

(b) recovering cytochrome c oxidase from the culture.

Claim 47. (Original) A process according to claim 46, wherein the recombinant microorganism is a bacteria.

Claim 48. (Currently amended) A process according to claim 46 47, wherein the microorganism bacteria is selected from the group consisting of *Escherichia coli*, *Pseudomonas putida*, *Acetobacter xylinum*, *Acetobacter pasteurianus*, *Acetobacter aceti*, *Acetobacter hansenii*, and *Gluconobacter oxydans*.

Claim 49. (Currently amended) A process according to claim 46 48, wherein the microorganism is obtained from *Gluconobacter oxydans* DSM 4025.

Claim 50. (Currently amended) A process according to claim 46 49, wherein the microorganism is a biologically and/or taxonomically homogeneous culture ~~biological or taxonomic homolog~~ of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.

Claim 51. (Withdrawn) A process for producing 2-keto-L-gluconic acid (2-KGA) from L-sorbose or D-sorbitol comprising:

(a) cultivating in a culture medium a recombinant microorganism comprising at least one polynucleotide or polynucleotide fragment selected from the group consisting a polynucleotide sequence of SEQ ID NO: 1, a polynucleotide fragment that encodes the amino acid sequence of SEQ ID NO: 2, a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 2, a polynucleotide fragment of SEQ ID NO: 3, a polynucleotide fragment that encodes the amino acid sequence of SEQ ID NO: 4, a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 4, the polynucleotide fragment of SEQ ID NO: 5, a polynucleotide fragment that encodes the amino acid of SEQ ID NO: 6, a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 6, a polynucleotide fragment of SEQ ID NO: 7, and a polynucleotide fragment that encodes the amino acid sequence of SEQ ID NO: 8, and a polynucleotide fragment that encodes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO: 8 and capable of expressing the complex in any one of claims 1 - 9 with cytochrome c oxidase activity; and

(b) recovering 2-KGA from the culture medium.

Claim 52. (Withdrawn) A process according to claim 51, wherein the recombinant microorganism is a bacteria.

Claim 53. (Withdrawn) A process according to claim 52, wherein the bacteria is selected from the group consisting of *Escherichia coli*, *Pseudomonas putida*, *Acetobacter xylinum*, *Acetobacter pasteurianus*, *Acetobacter aceti*, *Acetobacter hansenii*, and *Gluconobacter oxydans*.

Claim 54. (Withdrawn) A process according to claim 53, wherein the microorganism is *Gluconobacter oxydans* DSM 4025.

Claim 55. (Withdrawn) A process according to claim 54, wherein the microorganism is a biological or taxonomic homolog of a microorganism having the identifying characteristics of *Gluconobacter oxydans* DSM 4025.

Claim 56. (Withdrawn) A cytochrome c oxidase complex comprising a core subunit containing a polypeptide sequence selected from the group consisting of SEQ ID NO:2, 4, 6 and 8, fragments of SEQ ID NO:2 capable of providing the said complex with cytochrome c oxidase activity, and a polynucleotide sequence that encodes a polypeptide that is capable of providing the complex with cytochrome c oxidase activity, and which polynucleotide hybridizes under high stringency hybridization and wash conditions to a polynucleotide sequence encoding SEQ ID NO:2, 4, 6 or 8.

Claim 57. (Currently amended) ~~A recombinant DNA that encodes at least a part of core subunit I of a cytochrome c oxidase complex and that conveys cytochrome c~~

~~oxidase activity to the complex when present, the recombinant DNA~~ comprising a polynucleotide sequence that hybridizes to the complementary strand of SEQ ID NO: 1 under high stringency conditions comprising ~~[[()]]~~overnight incubation in 6X SSC, 0.5% SDS, 100 ug/ml denatured salmon sperm DNA, 50% formamide, ~~with gentle rocking~~ at 42°C; followed by a first wash in 2X SSC, 0.5% SDS at room temperature for 15 minutes; followed by a second wash in 0.1X SSC, 0.5% SDS at room temperature for 15 minutes ~~[[()]]~~, wherein the recombinant DNA encodes a polypeptide that forms a complex having cytochrome c oxidase activity with a *Gluconobacter oxydans* cytochrome c oxidase core subunit II.

Claims 58 and 59. (Cancelled).

Claim 60. (Currently amended) A recombinant DNA according to claim 24, wherein the complex comprises ~~is isolated from a *Gluconobacter oxydans* DSM 4025~~ cytochrome c oxidase core subunits I and II microorganism.

Claim 61. (Currently amended) A recombinant DNA according to claim 24, wherein the complex comprises ~~has the following properties:~~

(a) at least a core subunit I (COI) and a core subunit II (COII), wherein the apparent molecular masses of COI and COII are about  $43 \pm 10$  kDa and  $36 \pm 10$  kDa, respectively by SDS-PAGE~~[[;]]~~ and

(b) the complex displays an absorption spectrum showing an aa3-type cytochrome c oxidase peak at  $605 \pm 1$  nm in a reduced minus oxidized difference spectrum.

Claim 62. (Previously presented) A recombinant DNA according to claim 24, wherein the complex is a recombinant enzyme.

Claim 63. (Currently amended) A recombinant DNA according to claim 24, wherein core subunit II ~~the complex comprises at least one amino acid sequence selected from the group consisting of SEQ ID NO: 4, 6, or 8, and amino acid sequences that are 85% identical to SEQ ID NO: 4, 6 or 8.~~

Claim 64. (Currently amended) A recombinant DNA according to claim 26 ~~27~~, wherein the subunits are ~~complex is isolated from a~~ *Gluconobacter oxydans* DSM 4025 cytochrome c oxidase subunits ~~microorganism~~.

Claim 65. (Currently amended) A recombinant DNA according to claim 26 ~~27~~, wherein the complex comprises ~~has the following properties:~~

(a) at least a core subunit I (COI) and a core subunit II (COII), wherein the apparent molecular masses of COI and COII are about  $43 \pm 10$  kDa and  $36 \pm 10$  kDa, respectively by SDS-PAGE[[:]] and

(b) the complex displays an absorption spectrum showing an aa3-type cytochrome c oxidase peak at  $605 \pm 1$  nm in a reduced minus oxidized difference spectrum.

Claim 66. (Currently amended) A recombinant DNA according to claim 26 27, wherein the complex is a recombinant enzyme.

Claim 67. (Currently amended) A recombinant DNA according to claim 26 27, wherein core subunit I ~~the complex~~ comprises at least one amino acid sequence selected from the group consisting of SEQ ID NO: 2 and amino acid sequences that are 85% identical to SEQ ID NO: 2.

Claim 68. (Currently amended) A recombinant DNA according to claim 29 30, wherein the subunits are ~~complex is isolated from a~~ *Gluconobacter oxydans* DSM 4025 cytochrome c oxidase subunits microorganism.

Claim 69. (Currently amended) A recombinant DNA according to claim 29 30, wherein ~~the complex has the following properties:~~

(a) ~~at least core subunit I (COI) and core subunit II (COII), wherein the~~ apparent molecular masses of core subunits I and II ~~COI and COII~~ are about  $43 \pm 10$  kDa and  $36 \pm 10$  kDa, respectively by SDS-PAGE[[:]] and



(b) the complex displays ~~providing~~ an absorption spectrum showing an aa3-type cytochrome c oxidase peak at  $605 \pm 1$  nm in a reduced minus oxidized difference spectrum.

Claim 70. (Currently amended) A recombinant DNA according to claim 29 ~~30~~, wherein the complex is a recombinant enzyme.

Claim 71. (Currently amended) A recombinant DNA according to claim 29 ~~30~~, wherein core subunit I ~~the complex~~ comprises at least one amino acid sequence selected from the group consisting of SEQ ID NO: 2 and amino acid sequences that are 85% identical to SEQ ID NO: 2.

Claim 72. (Currently amended) An expression vector comprising at least one recombinant DNA according to any one of claims 60, 64 and 68 ~~claim 32~~, wherein the complex is isolated from a *Gluconobacter oxydans* DSM 4025 microorganism.

Claim 73. (Currently amended) An expression vector comprising at least one recombinant DNA according to any one of claims 61, 65 and 69 ~~claim 32~~, wherein the complex has the following properties:

(a) ~~at least core subunit I (COI) and core subunit II (COII), wherein the apparent molecular masses of COI and COII are about  $43 \pm 10$  kDa and  $36 \pm 10$  kDa, respectively by SDS PAGE; and~~

~~(b) — an absorption spectrum showing an aa3-type cytochrome c oxidase peak at  $605 \pm 1$  nm in a reduced minus oxidized difference spectrum.~~

Claim 74. (Currently amended) An expression vector comprising at least one recombinant DNA according to any one of claims 62, 66 and 70 ~~claim 32~~, wherein the ~~complex is a recombinant enzyme.~~

Claim 75. (Currently amended) A recombinant microorganism comprising at least one recombinant DNA according to any one of claims 60, 64 and 68 ~~claim 41~~, wherein the ~~complex is isolated from a *Gluconobacter oxydans* DSM 4025 microorganism.~~

Claim 76. (Currently amended) A recombinant microorganism comprising at least one recombinant DNA according to any one of claims 61, 65 and 69 ~~claim 41~~, wherein the ~~complex has the following properties:~~

~~(a) — at least core subunit I (COI) and core subunit II (COII), wherein the apparent molecular masses of COI and COII are about  $43 \pm 10$  kDa and  $36 \pm 10$  kDa, respectively by SDS-PAGE; and~~

~~(b) — an absorption spectrum showing an aa3-type cytochrome c oxidase peak at  $605 \pm 1$  nm in a reduced minus oxidized difference spectrum.~~

Claim 77. (Currently amended) A recombinant microorganism comprising at least one recombinant DNA according to any one of claims 62, 66 and 70 ~~claim 41~~, wherein the ~~complex is a recombinant enzyme.~~

Claim 78. (Currently amended) A process for producing a cytochrome c oxidase complex comprising: ~~according to claim 46, wherein the complex is isolated from a *Gluconobacter oxydans* DSM 4025 microorganism~~

(a) cultivating in a culture medium a recombinant microorganism according to claim 75; and

(b) recovering cytochrome c oxidase from the culture.

Claim 79. (Currently amended) A process for producing a cytochrome c oxidase complex comprising: ~~according to claim 46, wherein the complex has the following properties:~~

(a) cultivating in a culture medium a recombinant microorganism according to claim 76 ~~at least core subunit I (COI) and core subunit II (COII), wherein the apparent molecular masses of COI and COII are about  $43 \pm 10$  kDa and  $36 \pm 10$  kDa, respectively by SDS-PAGE; and~~

(b) recovering cytochrome c oxidase from the culture ~~an absorption spectrum showing an aa3-type cytochrome c oxidase peak at  $605 \pm 1$  nm in a reduced minus oxidized difference spectrum.~~

Claim 80. (Currently amended) A process for producing a cytochrome c oxidase complex comprising: ~~according to claim 46, wherein the complex is a recombinant enzyme~~

Application No.: 09/712,768  
Amendment Dated: November 11, 2000  
Reply to Office Action mailed: February 7, 2003

(a) cultivating in a culture medium a recombinant microorganism  
according to claim 77; and

(b) recovering cytochrome c oxidase from the culture.